A METHOD OF STANDARDISING PITUITARY (INFUNDIBULAR) EXTRACTS

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(Reprinted from the "Journal of Pharmacology and Experimental Therapeutics" Vol. IV. No 1, September, 191-)

Reprinted from THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
VOL. IV, No. 1, September, 1912

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From the Wellcome Physiological Research Laboratories, Herne Hill, London, S. E.

Received for publication, August 15, 1912

During recent years the watery extract or decoction of the posterior or infundibular lobe of the pituitary body has acquired wide use and importance in therapeutics, and seems likely to take its place among drugs of recognised and definite efficiency. The knowledge concerning the chemical nature of its active principle (or principles) is of the scantiest description, and there seems no prospect at present of the control of its efficiency by chemical methods. At the same time what is known indicates that the principle is of a kind readily destroyed by incipient putrefaction, the action of proteolytic ferments, or hydrolytic agencies in general. It is of considerable importance, therefore, to be able to control the activity of extracts intended for therapeutic use. The therapeutic applications of the extract are almost all based on the activities demonstrated by physiological experiment. Thus it is used for its properties of raising blood-pressure by arterial constriction (Oliver and Schäfer[1]), accelerating diuresis (Magnus and Schäfer,[2] Schäfer and Herring[3]), exciting contraction of the uterus (Dale,[4] Bell and Hick,[5] v. Fränkl Hochwart and Fröhlich[6]). Blair Bell, who gave the extract its first thorough clinical trial, observed in addition its power of restoring peristal-

[1] Oliver and Schäfer: Journ. of Phys., xviii, p. 277, 1895.
[2] Magnus and Schäfer: Ibid., xxvii, Proc. Phys. Soc., p. ix, 1901.
[3] Schäfer and Herring: Phil. Trans., 1906 B.
[4] Dale: Biochem. Journ., iv, p. 427, 1909.
[5] Bell and Hick: Brit. Med. Journ., i, p. 777, 1909.
[6] v. Frankl-Hochwart and Fröhlich: Arch. f. exp. Pathol. u. Therapie, lxiii, p. 347, 1910.

sis to the paralytically distended bowel, which is not represented by any definite action on the bowel of the normal animal. The galactagogue action (Ott and Scott,[7] Schäfer and Mackenzie[8]) may also prove to be therapeutically valuable.

In choosing, among these different expressions of activity, a suitable basis for standardisation, we are met at the outset by the uncertainty as to whether they are due to one or several principles. Schäfer and Herring pronounced definitely for a diuretic principle distinct from that responsible for the pressor action, but others have found their evidence faulty on some points and inconclusive on others. Again it has recently been stated by Engeland and Kutscher[9] that they succeeded in separating, by a method of fractional precipitation, from an extract of the whole pituitary body, a basic fraction possessing the characteristic action on the uterus, but not that on the blood-pressure. Judging from their records of the effect of the original extract on the blood-pressure they appear to have worked under conditions very little suited to the demonstration of that effect. A dose corresponding to 5 cc. of their original extract caused a small rise of blood-pressure in a rabbit, and a larger fall of pressure in a cat. On the other hand, a not exactly indicated quantity of the basic fraction produced some effect on a cat's isolated uterus, while approximately twice the amount of the same preparation had no perceptible action on the rabbit's blood-pressure and caused a slight fall of pressure in a cat. It does not seem to us that such qualitative evidence, which ignores the much greater sensitiveness of the uterine than the blood-pressure response, seriously affects the question whether the specific pituitary principles, causing rise of blood-pressure and uterine contraction, are identical or distinct. The theoretical possibility that each one of the different actions is due to a separate principle will, however, obviously remain open until a pure principle can be isolated, when it will be possible to discover how much of the activity of the whole extract it possesses. But the

[7] Ott and Scott: Proc. Soc. exp. Biol., New York, 1910.

[8] Schäfer and Mackenzie: Proc. Roy. Soc. B., lxxxiv, 1911; Mackenzie: Quart. Journ. exp. Physiol., iv, p. 305, 1911.

[9] Engeland and Kutscher: Zeit. f. Biol., lvii p. 527, 1912.

point of practical importance for purposes of standardisation is that in the ordinary extract the different types of activity seem to run strictly parallel. That is to say, we have never found an extract which was inferior in pressor action and had not a like inferiority as a diuretic and a uterine stimulant; nor have we succeeded in obtaining evidence of any treatment of the extract, deliberate or accidental, which weakens or destroys one action without weakening all in like degree. It seems safe, then, to adopt, as a criterion of the general activity of the extract, that one of its actions which can be measured with the greatest accuracy, whatever be the ultimate conclusion as to the number of principles involved."

At first sight the action on the blood-pressure, which has furnished by far the most reliable method of standardising suprarenal preparations, would seem the obvious choice. It has, however, very serious disadvantages in the case of pituitary extract. In the first place, the effect is apparently not a pure one, as Schäfer and Vincent[10] indicated.

It may be doubted whether the depressor action, seen with injections subsequent to the first, is wholly due to a different principle. Paton and Watson[11] have recently shown that in the duck a pure fall of blood-pressure is the characteristic effect of the extract. In any case, however, the waning of the pressor action (Howell[12]) and the increasing prominence of the preliminary depression, with injections subsequent to the first, constitute a very serious drawback to the use of blood-pressure effects as a method of comparing the action of two extracts on the same animal. On the other hand, the degree of pressor effect produced, in different individuals of the same species, by equal injections of the same extract, varies so widely, that no method of evaluation can safely be based on it. The difficulty of the declining effect of successive doses in the same animal may to a certain extent be overcome. If a cat with the cord cut in the neck and the brain destroyed be used, no injection being made until, under the artificial respira-

[10] Schäfer and Vincent: Journ. of Phys., xxv, p. 87, 1899.
[11] Paton and Watson: Journ of Phys., xliv, p. 413, 1912.
[12] Howell: Journ. exp. Med., iii, p. 2, 1898.

tion, all anesthetic has been removed, and the blood-pressure has become low and steady, the first dose, indeed, has an enormously greater effect than the second, while the third has yet smaller result. But if the doses given are small (not more than 0.1 cc. of a 10 per cent decoction of fresh tissue), it will be found that, after about the fourth dose, the effects of subsequent injections though small, are almost uniform. By interposing an injection of the preparation to be tested, at this stage, between two of an extract used as a standard of reference, a marked difference of activity can usually be detected. As indicating the scale of accuracy obtainable, we may say that we could usually rely on seeing an obvious difference between the effects of 0.2 cc. and 0.15 cc. of the same preparation by this method. A closer accuracy might be attained in some experiments; but the method was uncertain, and we frequently had to repeat a standardisation on two or three cats before coming to a definite conclusion.

The diuretic action seems likewise unsuited to the comparison of different preparations. The tolerance produced by a first injection is, indeed, less marked than in the case of the blood-pressure response, as Schäfer and Herring pointed out; but with large doses it is still well-marked, and if small doses are used it may be difficult to distinguish genuine effects from the spontaneous variations of urinary flow which occur in almost any experiment, however constant the controllable conditions. We have not made any experiments on the galactagogue effect, and have no evidence for or against its constancy in relation to the other activities of the extract. It does not, however, seem a likely basis for quantitative comparison, apart from the trouble of securing a regular supply of cats in lactation.

In seeking for a method which would overcome or minimise the difficulty caused by tolerance, we were guided partly by consideration of the probable cause of the phenomenon. The fact that tolerance is absent or, at least, for practical purposes negligible, in the case of the pressor effect of adrenine, has been generally attributed to the ease with which that base is destroyed by oxidation. In the case of a less easily oxidisable substance, such as the pituitary active principle, the restoration of the tissue to its origi-

nal condition of responsiveness may be expected to depend largely on gradual elimination by the kidneys, which is known to take place. The substance is not very readily diffusible, so that the concentration in the circulating fluids is probably reduced but slowly. How far the disappearance of the principle depends on such excretion, and to what extent it is destroyed by tissue ferments, is not known. But it is clear that injection into the intact circulation does not afford theoretically ideal conditions for the rapid return of the tissue to its original condition. It seemed probable that a thin strip of isolated muscle, suspended in a large volume of Ringer's solution, which could be rapidly removed and replaced by a fresh volume, would have a much better chance of recovering its original condition by the rapid washing out of the principle, or the reduction of its concentration below the threshold of activity. We have tried various forms of plain muscle for the purpose, but the only one giving satisfactory results is that of the uterus, which one of us (Dale[13]) showed previously to be exquisitely sensitive to the action of the extract, whether in the body or treated as an isolated organ. Kehrer, who first described the use of the uterus as an isolated organ,[14] has also observed its response to pituitary extract, and Engeland and Kutscher[15] employed the same method in the investigation already mentioned. The isolated horn of the uterus of the non-pregnant cat gives fairly good results, responding with but little diminished contraction to a second dose after a first has been carefully washed away. It is subject, however, to inconvenient spontaneous slow variations of its average tonus, and is apt to acquire a disconcerting rhythm. The uterine horn of the young virgin guinea-pig is greatly superior in these respects, its natural tendency, when left undisturbed in the Ringer's solution, being to acquire a condition of complete relaxation, broken only by a small rhythm. It is very sensitive to the extract, which can, therefore, be given in very small doses, and normally relaxes with promptitude to its original level of minimal tonus on washing out and changing the solution.

[13] Dale: Biochem. Journ., iv, p. 427, 1909.
[14] Kehrer: Arch. f. Gynäkol., lxxxi, p. 160, 1906.
[15] Engeland and Kutscher: Zeit. f. Biol., lvii, p. 527, 1912.

We have used it now for over a year for standardising pituitary extracts, with results which, though not ideal, seem to us an advance on those obtainable by other methods.

II. DESCRIPTION OF THE METHOD

The following is a description of the arrangement which we have had in use now for some years and have found convenient in this and similar experiments, involving the use of an organ isolated in Ringer's solution, the latter being renewable when necessary without disturbing the organ or the record.

The Ringer's solution in which the organ is suspended is contained in a vessel formed of a wide lamp glass (Fig. 1, *A*) such as is used with an incandescent gas-mantle. The lower end of this is plugged with a rubber bung (C_1) having one central bore. Through the latter passes one limb of a wide glass T-tube (T_1) which ends flush with the upper surface of the bung, so that the fluid in the lamp-glass can be run out through it to the last drop. This tube passes through a second rubber cork (C_2) which fills an opening in the bottom of an outer cylindrical copper vessel (*B*) which forms a constant-temperature water-jacket. The temperature is kept uniform by a device due to Locke, a copper rod (*R*) passing through the water-bath, the openings where it passes the walls being made tight with solder. On this rod hangs a Bunsen burner with a brass chimney, and, by shifting this burner along the rod when necessary, the temperature can easily be kept between 38° and 39°C. The other limbs of the T-piece (T_1) are connected by rubber junctions armed with spring-clips (*X*, *X*) to a waste-pipe, through which the lamp-glass is emptied, and a syphon tube (*S*) leading from a large flask which holds warm Ringer's solution for refilling. At the lower end of the syphon another T-piece (T_2) is interposed, which serves as a by-pass, allowing the syphon to be filled with warmed Ringer immediately before a change is required. Into the lamp-glass chamber dips a narrow glass tube (*O*). This is turned at a right angle about half an inch from its lower end. The end is sealed into a blob; into this a platino-iridium pin (P[16])

[16] A broken platino-iridium syringe-needle serves admirably.

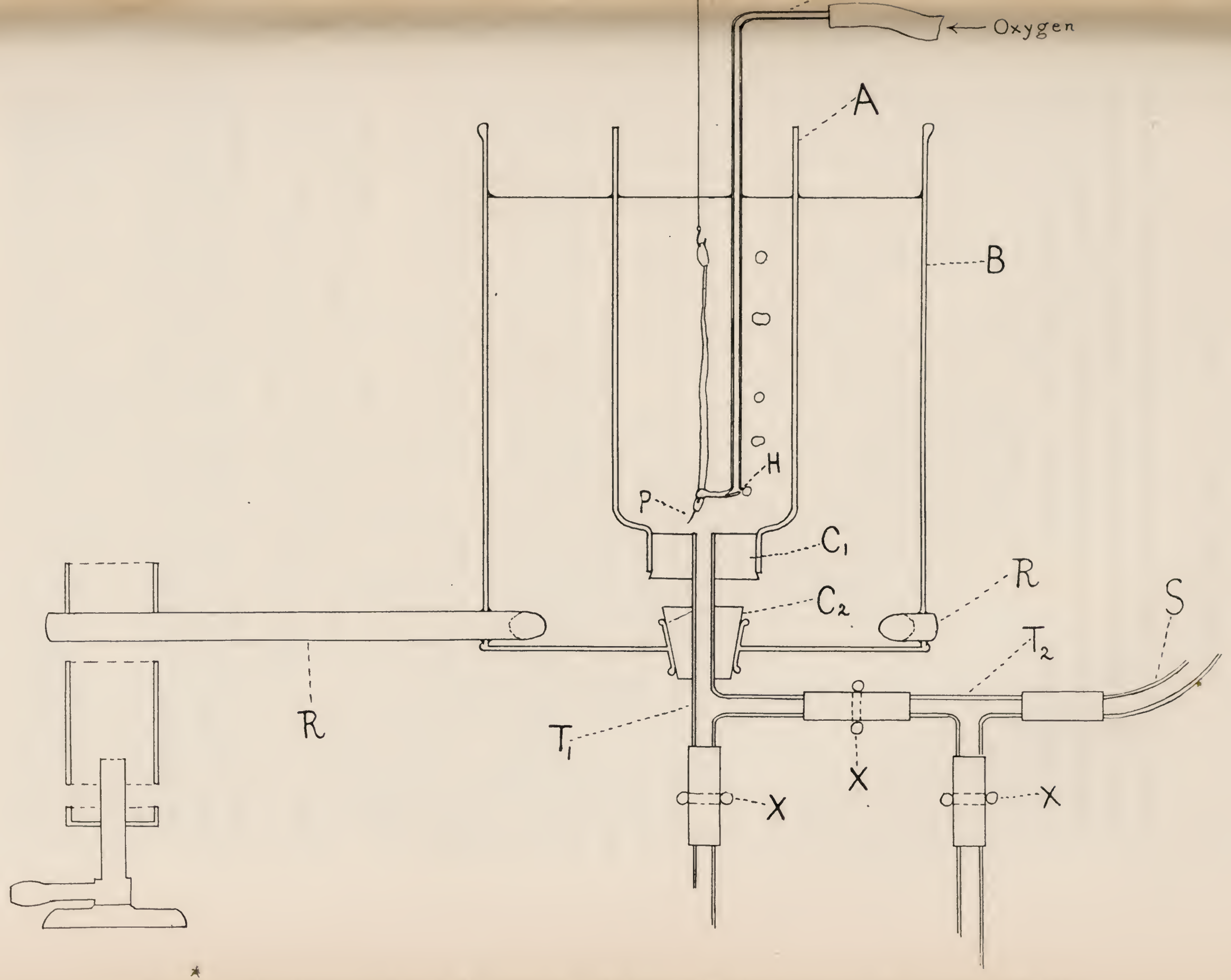

FIG. 1. DIAGRAMMATIC SECTION OF CONSTANT TEMPERATURE BATH FOR ISOLATED ORGANS (SEE TEXT)

is fused. This serves for attaching the lower end of the isolated organ. The upper end of the tube is connected to a bomb of oxygen, and the gas issues through a small hole (*H*) blown at the bend near the lower end of the tube. A continual stream of small oxygen bubbles thus rises through the Ringer's solution, which it oxygenates and stirs at the same time. The hole is so situated that the bubbles do not play directly on the suspended organ. Through the upper end of the latter is passed a small hook, which we usually make from a gilt entomological pin. This is attached by a silk thread to the shorter arm of a lever. The lever is made of a straw cemented to the side of an ebonite pulley, the axle of which furnishes the fulcrum. We generally use an ink writing point, a long paper being used and a flat surface secured by use of two small drums at the recording end of the kymograph (Fig. 2). This is essential for tracing with uniform pressure the long, sweeping curves drawn by contraction of the guinea-pig's uterus, even if a non-magnifying lever is used. A very light load is used and any variation of friction on the drum may disturb the result seriously. Over the pulley to which the lever is attached is fixed a little rubber-faced brake, controlled by a small Bowden cable, as supplied for actuating the release-mechanism of photographic shutters. This serves to fix the lever when required, so that the fluid can be changed without seriously interrupting the record. The ratio between lever-arms which we have usually employed magnifies the contraction about twice.

The temperature of the fluid in the reservoir flask is adjusted by periodical use of a burner beneath and by running in cold Ringer when needed. Some trouble might be saved by having a large reservoir of Ringer's solution in a thermostat, but this is undesirable for another reason. The solution which we use gradually loses CO_2 when kept at 38° to 40°C. for any length of time and deposits some of its calcium as carbonate. We shall see that this weakening of the calcium content is specially undesirable for our present purpose. It is better, therefore, to warm a moderate quantity at a time, keeping the main bulk cold. If the Ringer in the reservoir is kept at 40°C., and the cooled solution in the syphon replaced by use of the by-pass, the fluid reaches the experimental

bath at a temperature of 38° to 39°C. The solution we have used throughout is made up on Locke's formula.[17] The water used was distilled with a glass condenser. Having at one period a series of unsatisfactory results, the sensitiveness of the uterus in several

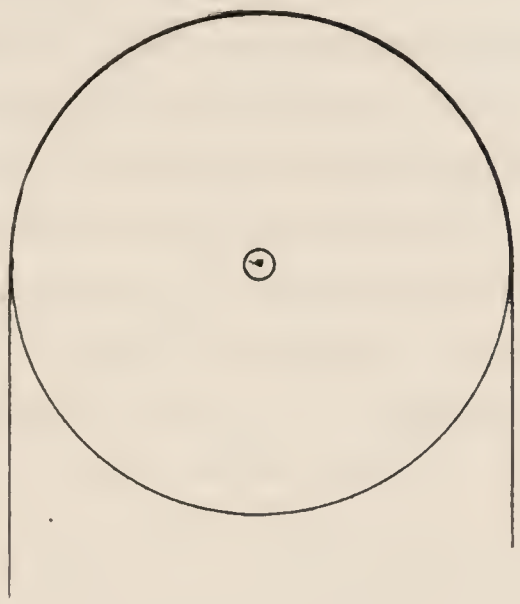

FIG. 2. KYMOGRAPH WITH FLAT WRITING SURFACE

experiments showing a rapid decline with successive doses, we investigated the composition of the Locke-Ringer solution, and discovered that, through an error in calculation, the batch in

[17] NaCl 9.0
KCl 0.42
$CaCl_2$ 0.24
Dextrose 1.0
$NaHCO_3$ 0.5
} in 1 litre of glass-distilled water.

use had been made up with only half the correct proportion of calcium chloride. On making up a new batch correctly we again obtained the almost uniform response to successive equal doses which is the chief advantage of the method. It is clear, then, that an adequate calcium content of the bathing fluid is an important factor in restoring the sensitiveness of the uterus after an effective dose of the extract. The volume we have used in the bath is 250 cc., with which the lamp-glass chamber which we use is about three-quarters filled. The water in the outside jacket is adjusted to stand at the same level, and serves as a guide to the refilling of the bath with the same volume of fluid at each change.

Young virgin guinea-pigs, of not more than 350 grams weight, usually give the best results. With older animals the uterus is apt to show too much automatic rhythm and to be too sensitive, though it may occasionally give an easily interpreted record. The animal is killed by a blow on the head and bled out as completely as possible. Some experiments undertaken for another purpose suggest that more uniformly good results might be obtained by perfusing the uterus thoroughly with the Ringer's solution, through the aorta, before excision; but the procedure is probably not worth the trouble involved for routine use. All the results dealt with in this paper have been obtained with the organ simply excised and suspended after bleeding the animal. A horn of the uterus is removed by cutting through the broad ligament close to it along its entire length. The incision is carried forward so as to excise the ovary as well, which is left attached to the uterus by the fold of broad ligament in which the Fallopian tube runs. The pin at the lower end of the oxygen-tube is pushed through the lower end of the horn, and the hook suspended from the recording lever is passed through the ovary. The oxygen-tube is then lowered into the bath of Ringer's solution, to such a depth that the lever is pulled well beyond the horizontal position. The manipulation and exposure, followed by immersion in the warm bath, will be found almost invariably to produce a high degree of tonus. When the uterus is left to itself the tone slowly gives way, with small rhythmic interruptions, until a condition of almost complete relaxation is attained. The ideal uterus for our

purpose shows small rhythmic contractions rising from a steady level of minimum tonus. If the uterus is rather large and the rhythm excessive the load on the lever may be increased by a small weight. For the very slender horns of young virgin uteri the excess weight of the longer arm of the straw lever, with the brass ink-writing point at its tip, has usually been found a sufficient load and has needed partial counterpoising in some cases.

There seems to be some difference in the automatic activity of the uterus at different times of the year, and we have found it, on the whole, more quiescent, and more regularly responsive to the pituitary extract during the winter months. At the same time quite good results are obtainable during the summer months; all the tracings reproduced in illustration here were obtained during this July.

When the uterus has attained the condition of uniform low tonus, which usually takes from fifteen to thirty minutes after suspension in the bath, the first dose of pituitary extract is added. The dose should be chosen so as to produce a nearly, but not quite maximal tonus, the lever rising with a steady sweep, broken only by slight rhythmic pauses or trifling relaxations. If, after rising to a position of partial tonus, the lever falls again and a wide rhythm ensues, the dose is too small, or, in rare cases, the uterus is unsuitable for the test. A few trials may be necessary to determine the appropriate dose. We usually begin with 1/100 to 1/80 cc. of our standard extract, to which we shall refer again later. If that is not enough the Ringer's solution is changed and the uterus allowed to relax again. After five to ten minutes at minimal tonus a larger dose (1/50 or 1/40 cc.) is then tried. If this is not sufficient it is best to discard the uterus and put up another. Figure 3 shows two successive responses of a fairly good uterus to 1/80 cc. of the same extract. In rare cases this dose may be too large, i.e., produce repeated supramaximal responses. In that case smaller doses, e.g., 1/200 cc. are used. The dose having been found which gives the desired smooth rise to nearly maximal tonus the Ringer is changed, and after relaxation and the usual interval of normal tracing, the same dose of the preparation to be compared with the standard is added to the

bath. If this is approximately of standard strength the curve traced will be almost indistinguishable from that traced after the standard dose. On the other hand, if the preparation is definitely weaker than the standard, and if the dosage has been chosen

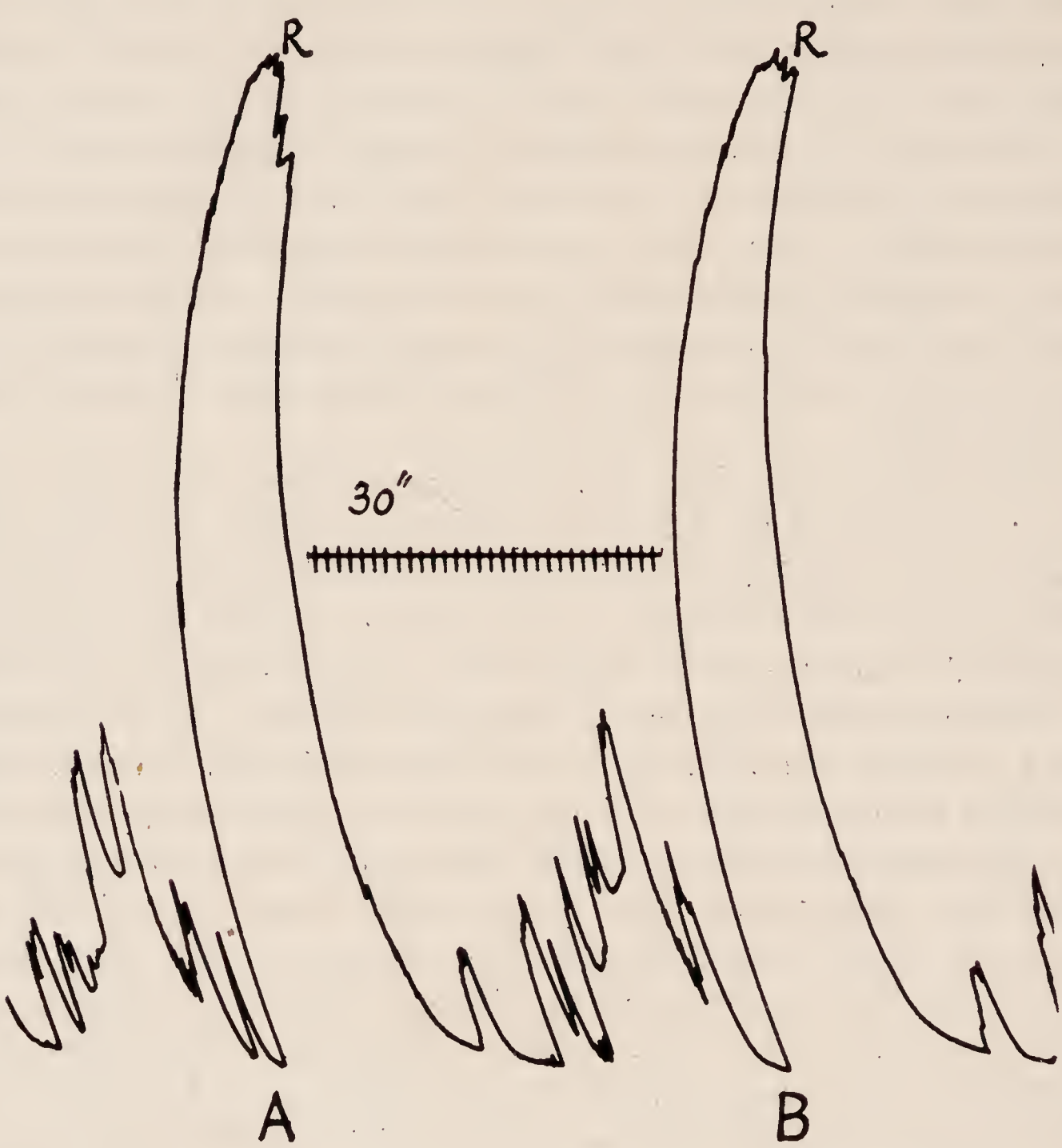

FIG. 3. THIS AND FOLLOWING FIGURES (EXCEPT FIG. 7) REPRESENT RECORDS FROM THE ISOLATED UTERUS OF THE VIRGIN GUINEA-PIG IN 250 CC. OF RINGER'S SOLUTION.

At *A*, 0.0125 cc. standard (20 per cent) infundibular extract added to the bath; at *B*, the same; at *RR* (in this and succeeding figures), change to fresh Ringer.

rightly, so as not to be supramaximal, the summit of the second curve will fall clearly below that of the first. If the second curve rises slightly higher or lower than the first, this is not necessarily

significant, since a second equal dose of the same preparation will frequently give a slightly bigger effect than the first, or may, on the other hand, cause a slightly smaller one. The uniformity of result is more complete with some uteri than others, and in almost all cases tends to increase with doses succeeding the second. It depends to a considerable extent on the uniformity of interval

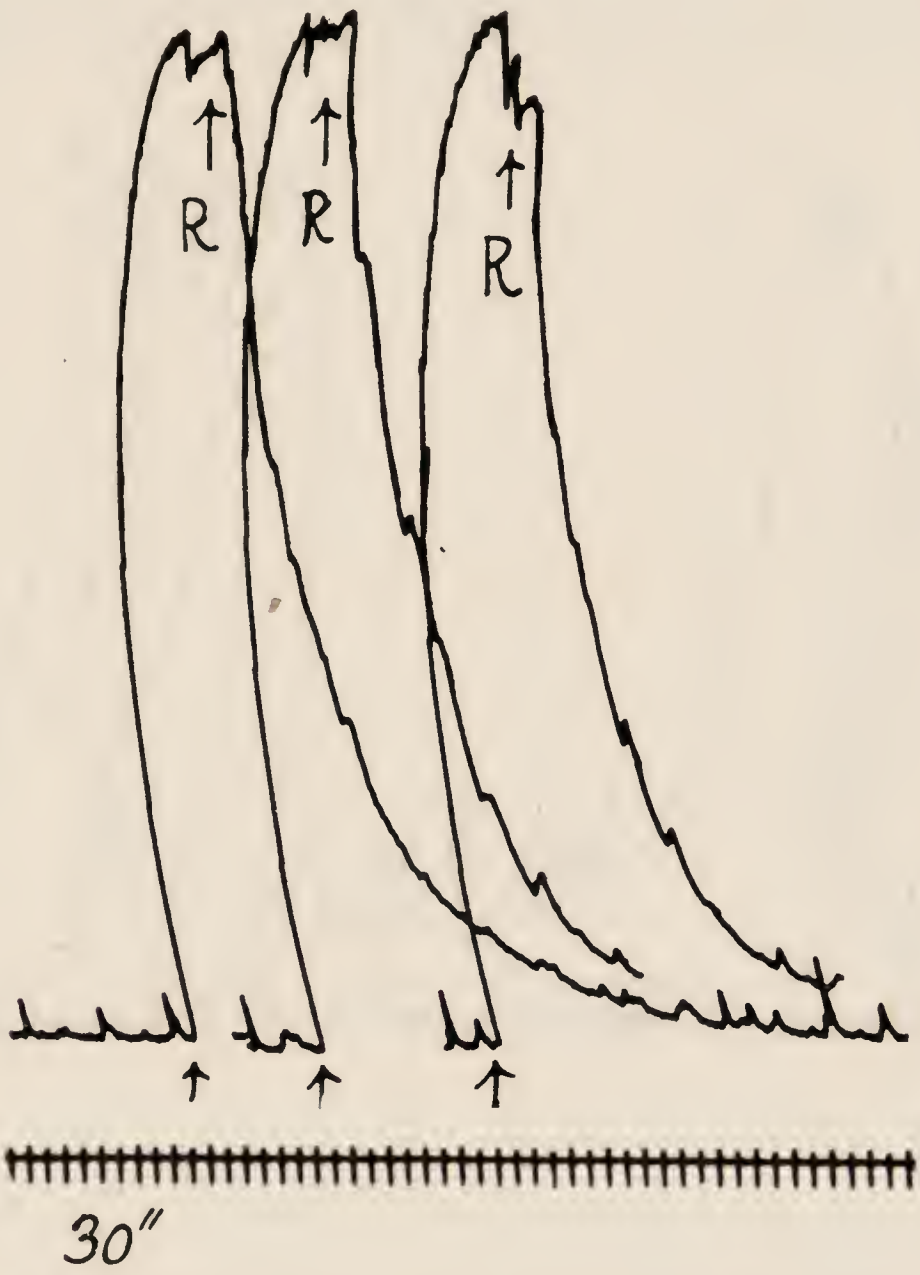

FIG. 4. THREE SUCCESSIVE RESPONSES TO 0.025 CC. OF THE SAME EXTRACT, OVERLAPPED FOR COMPARISON

between the doses, so that the first dose of a group, either at the beginning of the experiment, or at a later stage following a prolonged interruption, tends to produce a slightly abnormal result in one direction or the other. This can be seen in Figures 8 and 10 where the first of a group of doses, given after a prolonged interruption in each of these cases, produces a slightly greater contrac-

tion than the same dose later in the group, the intervals between the doses within the group being approximately ten minutes in each case. Figure 4 shows a group of three contractions produced by successive equal doses of the same preparation. It will be seen that the first dose of this series produces a slightly smaller effect than either the second or third—that is to say, the first dose after an interval in this case causes a temporary increase in the sensitiveness of the uterus. With a long series of doses at short

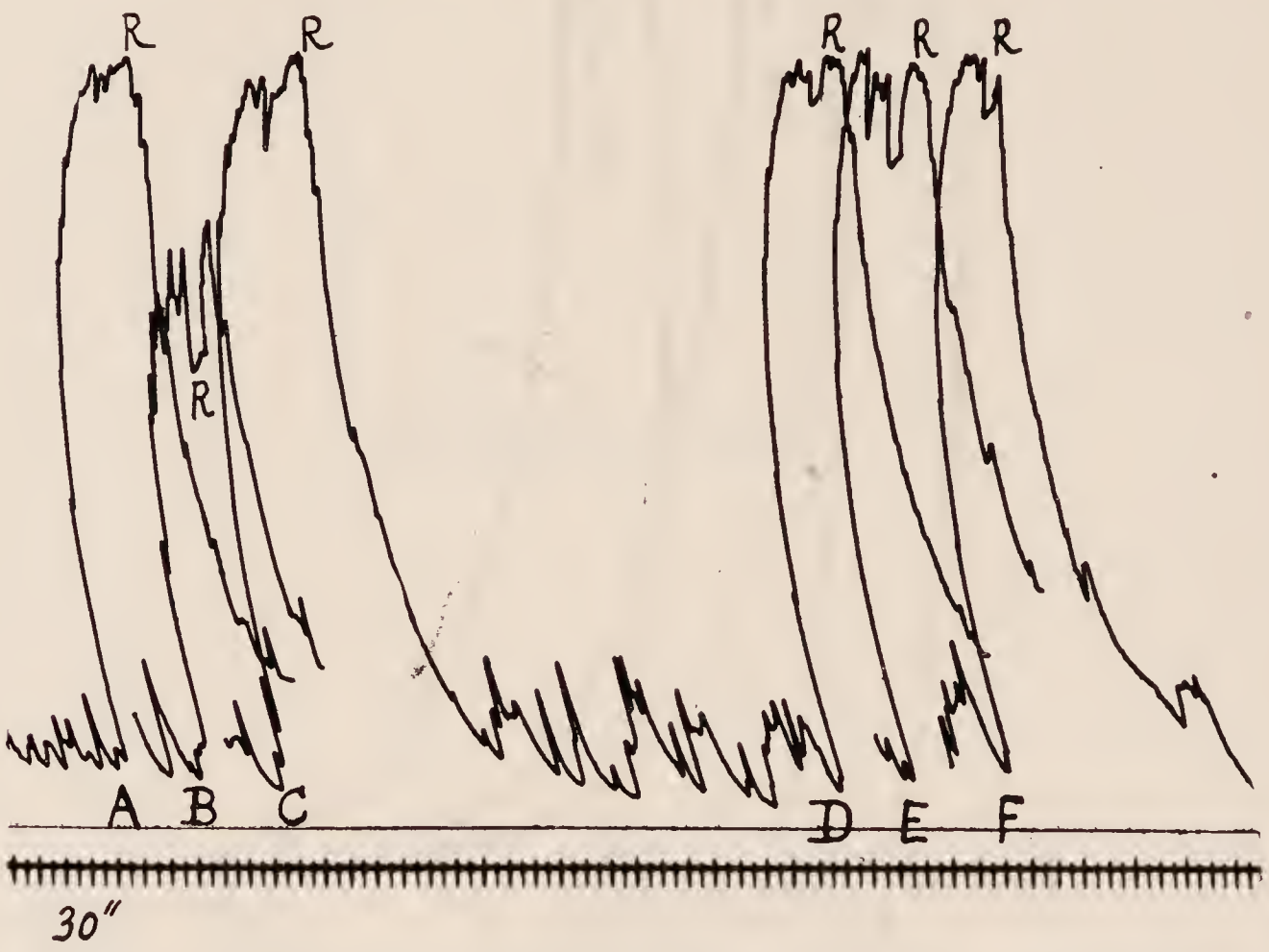

FIG. 5. COMPARISON OF EXTRACT T WITH STANDARD EXTRACT S

At *A*, 0.0125 cc. S; at *B*, 0.0125 cc. T; at *C*, 0.0125 cc. S; at *D*, 0.02 cc. T; at *E*, 0.0125 cc. S; at. F, 0.02 cc. T.

intervals a gradual decline in response takes place, and it is better, in performing a series of comparisons with the same uterus, to give a group of at most four or five doses at ten minute intervals, then allow an interval of twenty minutes or so and give another group, regarding the first member of each group of curves as probably abnormal. Uteri vary greatly as regards the length of time during which they give regular responses; with a good specimen reliable results can be obtained throughout an ordinary working

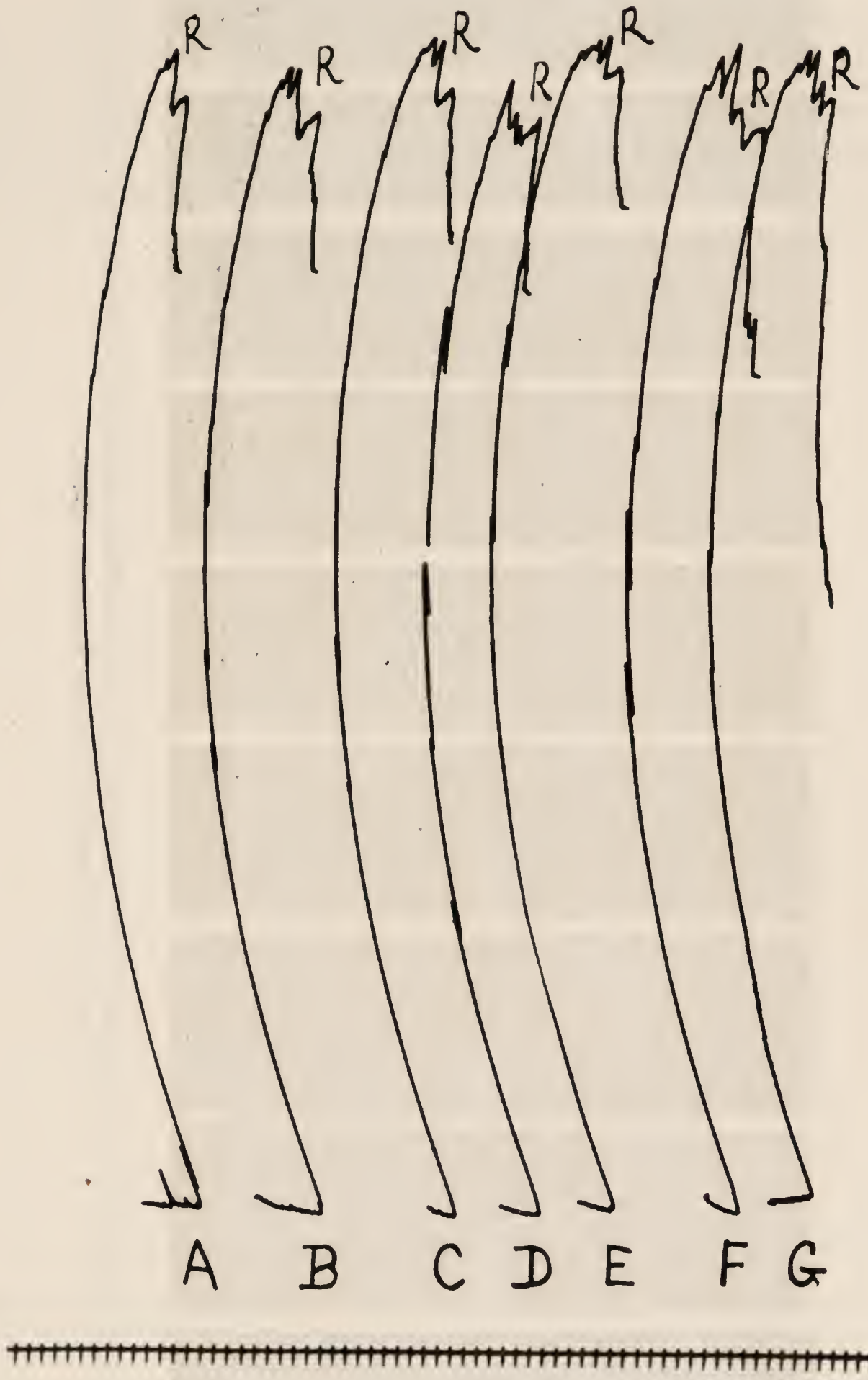

FIG. 6. THE SAME EXTRACTS AS IN FIGURE 5 COMPARED WITH ANOTHER (MORE HIGHLY SENSITIVE) UTERUS

At *A*, 0.0075 cc. S; at *B*, 0.0075 cc. T; at *C*, 0.0075 cc. S; at *D*, 0.0075 cc. T; at *E*, 0.0075 cc. S; at *F*, 0.005 cc. S; at *G*, 0.0075 cc. T.

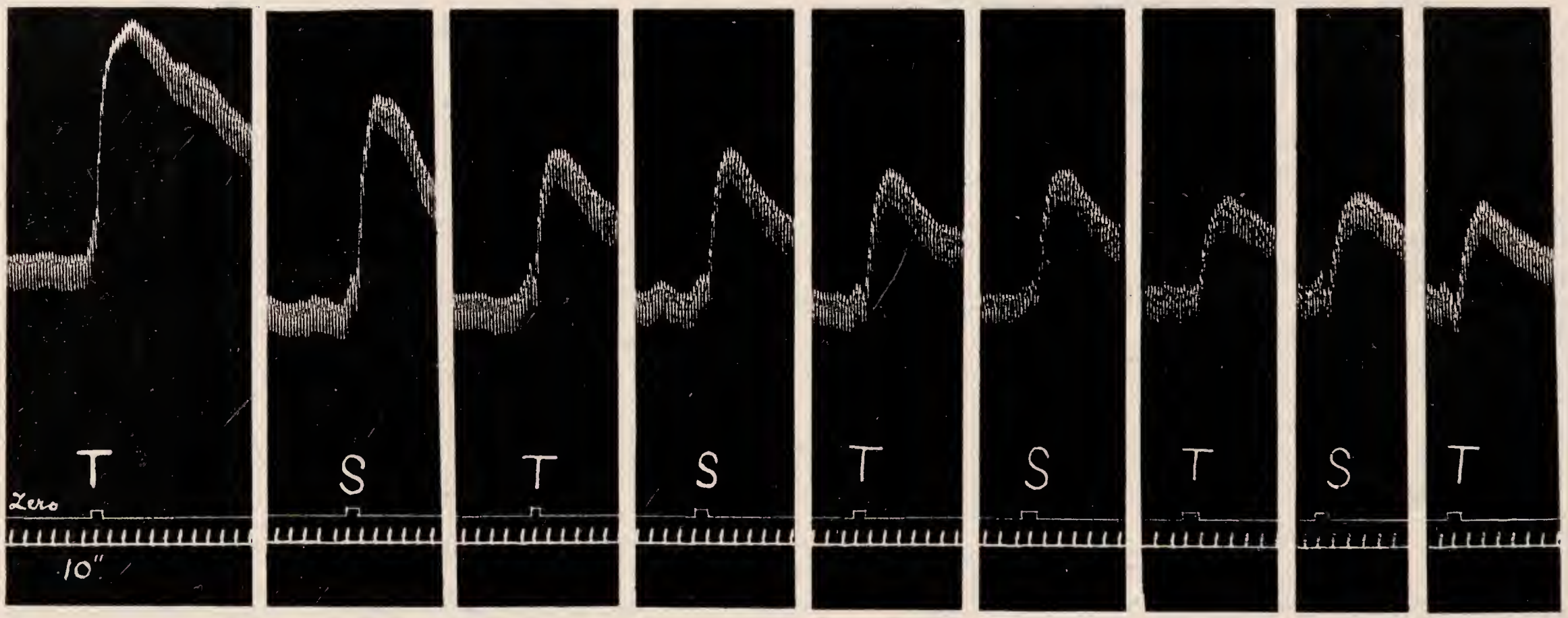

FIG. 7. THE SAME EXTRACTS AS IN FIGURES 5 AND 6 COMPARED ON THE BLOOD-PRESSURE OF A PITHED CAT. DOSE IN EACH CASE 0.05 CC. INJECTED BY THE FEMORAL VEIN

day. We have deliberately emphasized those sources of error which demand care and experience for their complete elimination. At the same time it should be made clear that the precautions mentioned above are only needed for the application of the method with a fineness of discrimination altogether beyond the range of

FIG. 8. COMPARISON WITH THE STANDARD (S) OF A NEARLY EQUIVALENT EXTRACT (X)

At *A*, 0.0125 cc. X (abnormal effect of first dose); at *B*, 0.0125 cc. S; at *C*, 0.0125 cc. X; at *D*, 0.0125 cc. S.

differences appreciable by other methods. In our experience a difference of activity which is only just appreciable by the blood-pressure method under the best conditions is at once obvious by the test on the uterus without any special care in controlling the regularity of the response. Such a case is shown in Fig. 5, which

illustrates the comparison with the standard of a preparation decidedly deficient in activity. It will be seen that the specimen (T) gives in equal doses with the standard (S) produces a very obviously smaller effect. It needs about 0.02 cc. of *T* to produce the same effect as 0.0125 cc. of S. On another more sensitive uterus (Fig. 6) the equivalent doses were found to be 0.0075 T to

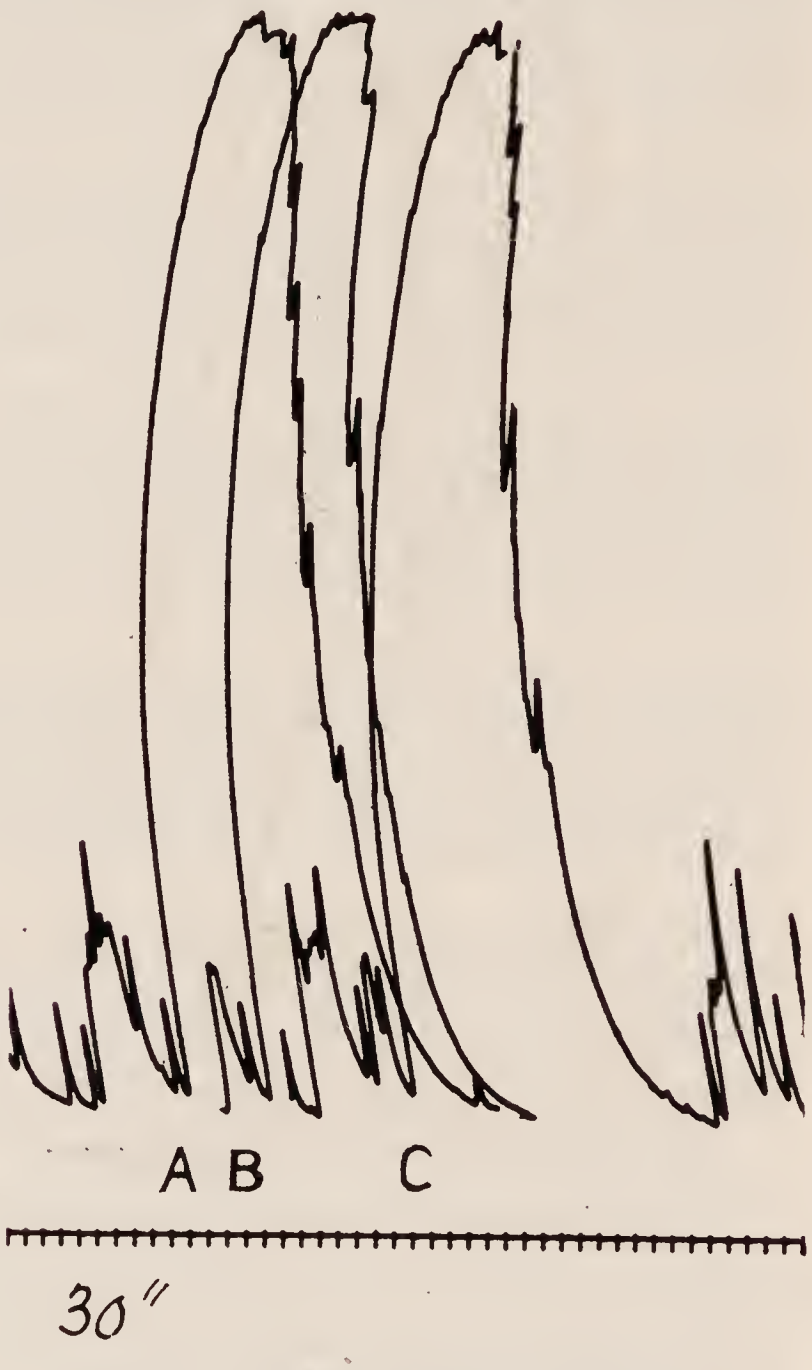

FIG. 9. CONTINUATION OF FIG. 8

At *A*, 0.0125 cc. S; at B, 0.014 cc. X; at *C*, 0.0125 cc. S

0.005 S. Certain limitations of the method, with the corresponding precautions desirable, can be gathered from inspection of Figure 6. It will be seen that the uterus which is highly sensitive to minute doses does not necessarily exhibit so great a discrimination between doses of different value as a less sensitive organ, such

as that which gave Figure 5. It will be seen, moreover, that the tendency of the highly sensitive uterus is to give increasing rather than diminishing responses to successive equal doses. By being careful to alternate the doses, however, and to compare each effect with that immediately preceding and succeeding it, the slow increase of sensitiveness is discounted, the inferiority of T made

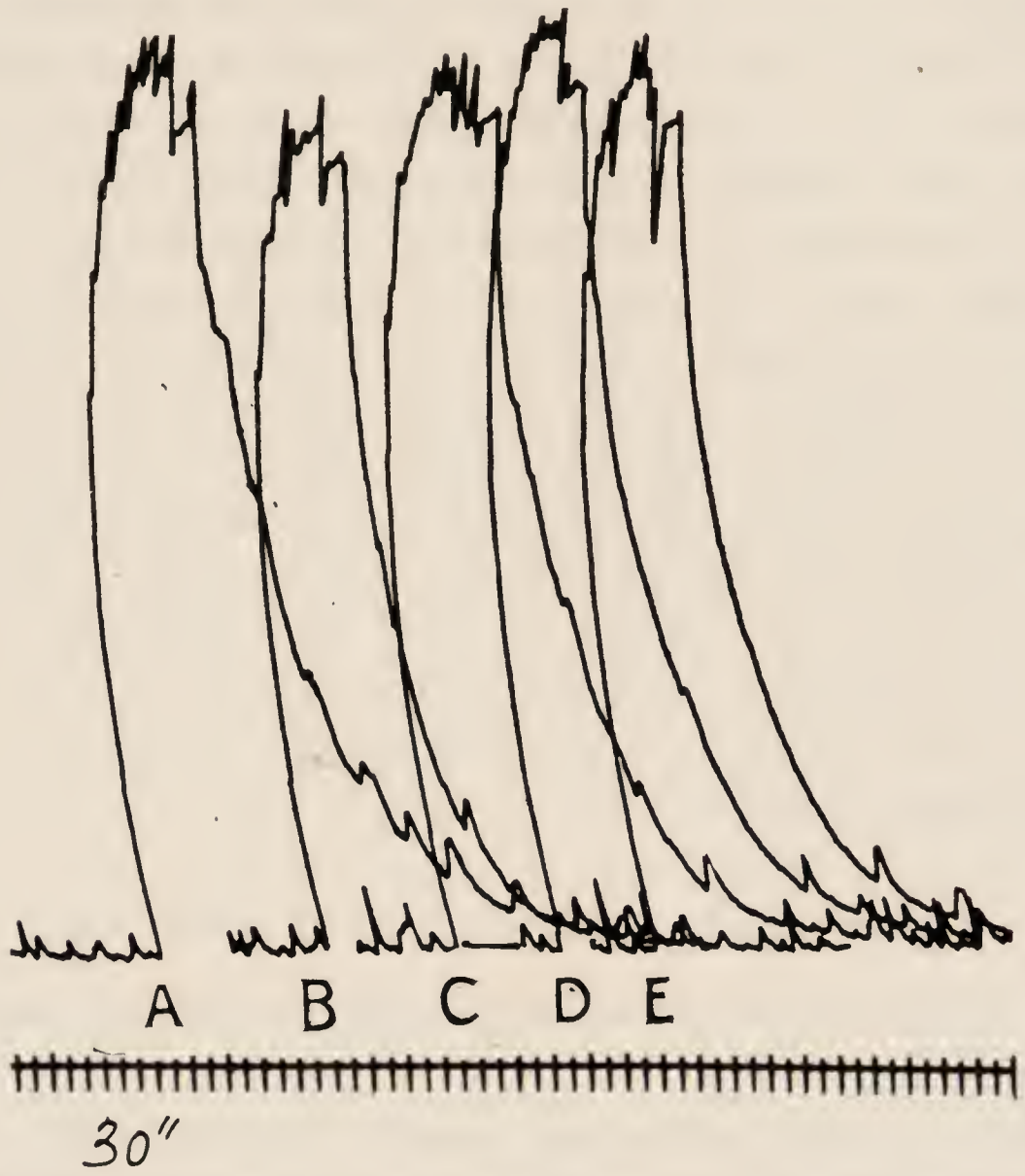

FIG. 10. VARYING DOSES OF THE SAME EXTRACT. UTERUS OF RATHER LOW SENSITIVENESS

At *A*, 0.025 cc. (abnormal effect of first dose); at *B*, 0.02 cc; at *C*, 0.025 cc; at *D*, 0.03 cc.; at *E*, 0.025 cc.

abundantly clear, and the approximate equivalence of 0.0075 T to 0.005 S established. This tracing shows the discriminating power of the method at less than its best and we have reproduced it for that reason. It is instructive to compare these results with the attempt to standardise the same preparation (T) against the same standard specimen (S) by the effect on the blood-pressure.

Figure 7 shows the declining pressor effects produced by alternating equal doses of the two. By careful comparison in the later stages it can be made out that the line joining two S summits always falls above the intervening T summit, and the conclusion may be drawn that T is probably inferior in activity. But it would be difficult by this method to get any quantitative idea of the deficiency: in fact it may be regarded as probable that T would be passed as corresponding sufficiently to the standard by any observer who was not aware, from comparative experience with the uterus method, that a deficiency recognisable at all by the blood-pressure test is probably of serious dimensions. Figures 8 and 9 illustrate the recognition of a much smaller difference in activity—practically a 10 per cent difference. Figure 10 illustrates the effect of a group of varying doses of the standard preparation, showing that, discarding the first contraction as abnormal, doses in the ratio 4:5:6 give very clearly differentiated effects. In this case the accuracy is, therefore, 20 to 25 per cent. As a rule we do not trouble to push the estimation to closer limits for standardising a preparation for practical use; but greater accuracy when required, as in renewing the standard, is attainable, as we have shown.

CHOICE AND MAINTENANCE OF A STANDARD

This is a point on which some generally acceptable convention is ultimately to be desired, but further experience of the conditions of stability is needed before any definite recommendation can be given. Schäfer and Herring state that the fresh infundibular substance, dried at low temperature, is indefinitely stable, and it may be that ultimately a freshly prepared decoction of such dried material will prove to be the best standard of reference. But Schäfer and Herring's statement was evidently based on general impression—the effects produced, that is to say, did not become obviously weaker on the average. The question is by no means a simple one, for no absolute standard of reference exists, on the one hand, which can be with certainty reproduced, while, on the other hand, the available methods of evaluation,

including the one we have described, are essentially comparative. The variation in the responsiveness of different individuals, or different isolated uteri, is very wide, so that neither the minimal effective dose nor the magnitude of response to a dose of standard size gives any useful information. For the present we used as a standard the extract prepared by a brief boiling of the perfectly fresh and finely pounded infundibular material with a definite proportion of acidulated water, so as to produce a 10 per cent or 20 per cent extract of the fresh moist substance. The extract is then sterilised by brief autoclaving in small phials. Some activity is lost in autoclaving, but the preparation thereafter has great stability. The use of small phials, of which one can be used for each test, obviates repeated sterilisation, which is inadmissible. At frequent intervals a new batch is chosen which has an activity equal to or just perceptibly greater than that of the former standard, so that the possibility of a very slow lowering of the standard is eliminated, though we have as yet no evidence of deterioration under such conditions.

SUMMARY

A method of comparative physiological estimation of the activity of pituitary (infundibular) extracts is described, the test organ being the isolated uterus of the virgin guinea-pig, which, when certain precautions are observed, is found to give a very uniform series of responses to successive equivalent doses. This method is found to detect differences of activity which escape recognition by the blood-pressure test, but appreciable differences of pressor activity always correspond to large differences of action on the uterus in the same direction. The action on the uterus may be adopted, therefore, as a criterion of the general activity of the extract. The method is essentially comparative and not absolute, so that an arbitrary standard must be adopted and renewed at intervals.